



NUTRIENT TRANSPORT IN STARFISH. II. UPTAKE OF NUTRIENTS BY ISOLATED ORGANS¹

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Evidence has been presented in a previous paper (Ferguson, 1964) that the coelomic fluid of starfish (*Asterias forbesi*) contains small but significant concentrations of amino acids and other materials which appear to be important in the process of nutrient translocation. These materials could be mobilized from the storage depots of the digestive glands and rapidly circulated in the coelomic fluid to other areas by the ciliary mechanisms described by Irving (1924) and Budington (1942). In order to verify this hypothesis, it is necessary to show that (1) nutrient materials may be released from the storage tissues into the coelomic fluid, (2) nutrient materials may be extracted from the coelomic fluid by the tissues requiring them, and (3) these operations may occur at rates sufficient to satisfy the metabolic needs of the animals.

These processes, common to most animals, may be quite readily studied in starfish. Several of the major organs of the body—the digestive glands, cardiac stomach, gonads, and rectal caeca—can be easily excised and maintained for considerable periods in clean sea water, which in composition is very similar to coelomic fluid (Cole, 1940). The extended survival of the organs under such conditions attests to the relatively autonomous existence they must normally lead. If the coelomic fluid is the medium of nutrient, waste, and gaseous transport, these preparations should approximate the actual conditions in the animals. In the present investigation, preparations of this type were used to confirm the occurrence of the coelomic transport mechanism of starfish, and to examine the properties and capabilities of this mechanism in nutrient translocation.

MATERIALS AND METHODS

The animals used in this work were freshly collected specimens of *Asterias forbesi* from the Woods Hole region, maintained in the laboratory in tanks supplied with adequate quantities of running sea water. Organs were carefully excised, rinsed in filtered sea water, and then placed in an incubative medium—either filtered sea water or cell-free coelomic fluid, extracted and pooled from several animals. Usually, organs weighing about 0.5 to 0.7 gm. were selected to be placed

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TABLE I
Uptake of nutrients by isolated tissues in sea water

Tissue	Nutrient	Weight gm.	Time hrs.	Init. Act. soln. $\mu\mu\text{c.}$	Final Act. soln. $\mu\mu\text{c.}$	Act. Removed $\mu\mu\text{c.}$	Final Act. Tissue $\mu\mu\text{c.}$
Rectal caeca	glucose	0.060	6	8,030 \pm 113	6,690 \pm 94	1,340 \pm 147	1,410 \pm 16
Rectal caeca	glucose	0.065	6	9,030 \pm 126	7,420 \pm 104	1,510 \pm 163	1,240 \pm 20
Rectal caeca	glycine	0.026	6	14,300 \pm 100	8,450 \pm 83	5,850 \pm 130	6,850 \pm 43
Rectal caeca	glycine	0.018	6	12,650 \pm 88	4,290 \pm 42	8,360 \pm 120	5,570 \pm 31
Gonads	glucose	0.190	8	17,950 \pm 172	5,510 \pm 55	12,440 \pm 181	10,700 \pm 43
Gonads	glycine	0.134	8	14,200 \pm 140	4,800 \pm 47	9,400 \pm 148	7,740 \pm 35
Stomach	glucose	0.090	3	1,280 \pm 13	890 \pm 10	390 \pm 16	290 \pm 3
Stomach	glucose	0.113	3	1,070 \pm 9	830 \pm 8	240 \pm 11	230 \pm 2

The organs were placed in 10 ml. of sea water together with small amounts of C^{14} -labeled nutrients. Absorption of the tracers by the tissues is indicated both by the disappearance of activity from the medium (Act. removed) and by subsequent demonstration of the activity in the tissues themselves (Final Act. Tissue). Error terms are based on the standard deviation of the counting rate.

in 25 ml. of medium to which a C^{14} -labeled nutrient had been added. When only small amounts of tissue could be obtained, as in the studies with rectal caeca, gonads, and stomach, only 10 ml. of medium were used. No attempt was made to maintain sterile conditions or to control pH. Irving (1926) has shown that the survival of digestive glands from *Patiria* placed in sea water is markedly affected by hydrogen ion concentration, but that the tissues will tend to modify the pH to their own optimum. He reports that digestive glands survive under such conditions for up to two days.

TABLE II
Uptake of nutrients by digestive glands in sea water

Nutrient	Time hrs.	Init. Act. soln. cpm.	Final Act. soln. cpm.	Act. removed by organ cpm.
Glucose	9	1,841 \pm 9	152 \pm 1.5	1,689 \pm 9
Glucose	9	1,951 \pm 14	140 \pm 1.4	1,810 \pm 14
Glucose	9	1,876 \pm 13	169 \pm 4.0	1,707 \pm 14
Glycine	9	3,280 \pm 23	54 \pm 1.0	3,226 \pm 23
Glycine	9	3,462 \pm 25	89 \pm 1.1	3,373 \pm 25
Glycine	9	3,432 \pm 24	135 \pm 4.3	3,297 \pm 24
APH*	9	4,319 \pm 25	344 \pm 2.5	3,965 \pm 25
APH*	9	4,216 \pm 25	596 \pm 3.0	3,620 \pm 24

* Algal protein hydrolysate.

This table shows the effect produced by isolated digestive glands when placed individually in beakers containing 25 ml. of sea water and a small amount of C^{14} -labeled material. The error terms are based on the standard deviation of the counting rate.

TABLE III
Uptake of nutrients by digestive glands in coelomic fluid

Nutrient	Time hrs.	Init. Act. soln. cpm.	Final Act. soln. cpm.	Act. removed by organ cpm.
Glucose	7	765 ± 5	53 ± 2	714 ± 5
Glucose	9	718 ± 5	54 ± 3	644 ± 6
Glycine	9	2,080 ± 17	557 ± 4	1,523 ± 17
Glycine	9	2,100 ± 17	524 ± 4	1,576 ± 17
APH*	8	1,607 ± 13	231 ± 3	1,376 ± 13
APH*	9	1,918 ± 19	252 ± 4	1,666 ± 19

* Algal protein hydrolysate.

This table shows the effect produced by isolated digestive glands when placed individually in beakers containing 25 ml. of pooled, cell-free coelomic fluid and a small amount of C¹⁴-labeled material. The error terms are based on the standard deviation of the counting rate.

The cultures were maintained at a constant temperature of 21° C. in a bath of running tap water. Air was gently bubbled through the medium to keep it circulating. Three kinds of tracers were used—(1) algal protein-C¹⁴ hydrolysate (essentially a mixture of amino acids with about 20% undetermined material), (2) glycine-1-C¹⁴, and (3) D-glucose-C¹⁴ (U.L.). In most of the experiments about 2.5 microcuries (μc.) were used in 25-ml. portions of medium, representing a concentration of nutrients approximating levels observed in normal specimens. Samples of the media (0.25 ml.) were drawn at intervals, diluted with 0.5 ml. of distilled water, and plated in duplicate 0.25-ml. aliquots on stainless steel planchets, which had been ringed with a wax pencil and coated with a spreading agent. The samples were counted on a Nuclear Chicago gas flow counter equipped with a "Micromil" window and an automatic sample changer. The quantity of tracer accumulated in some of the tissues was assayed at the end of the experiments by previously described methods (Ferguson, 1964).

In other experiments, total nitrogen and free ammonia nitrogen was measured

TABLE IV
Digestive glands in sea water with inhibitor

Nutrient	Time hrs.	Weight gm.	Init. Act. soln. cpm.	Final Act. soln. cpm.	Difference cpm.
Glucose	9	0.360	1,480 ± 15	1,350 ± 13	80 ± 20
Glucose	9	0.320	1,650 ± 15	1,520 ± 14	130 ± 21
Glycine	9	0.395	627 ± 9	495 ± 8	132 ± 12
Glycine	9	0.365	653 ± 10	474 ± 8	179 ± 13
Glycine	7	0.870	1,460 ± 14	1,160 ± 12	300 ± 18
Glycine	7	0.920	1,380 ± 14	1,078 ± 11	302 ± 18

These experiments were similar to those seen in Table II except that 2×10^{-4} M sodium iodacetate was added to the medium. The values for the activity removed (Difference) would be even lower except for self-absorption effects that occur in the samples. The error terms are based on the standard deviation of the counting rate.

in preparations containing 10 ml. of medium. Analysis of 2-ml. samples drawn from these was achieved by methods previously described (Ferguson, 1964). Inhibition of metabolic activity in some organs was induced with medium containing $2 \times 10^{-4} M$ sodium iodoacetate.

RESULTS

In these experiments the tissues remained in an apparently healthy condition for over 12 hours and frequently demonstrated spontaneous movements. All showed the ability to remove small amounts of labeled nutrients from the media (Tables I, II and III). This uptake was greatly affected by metabolic inhibition (Table IV). The inhibition was probably more complete than would be indicated by the table, for much of the slight apparent decrease in radioactivity of the medium was most likely due to the increasing self-absorption of the samples as organic materials accumulated in the medium during the experiments.

Absorption of glucose-C¹⁴

These experiments showed little difference in the ability of the digestive glands to remove low concentrations of labeled glucose from either sea water (Fig. 1) or

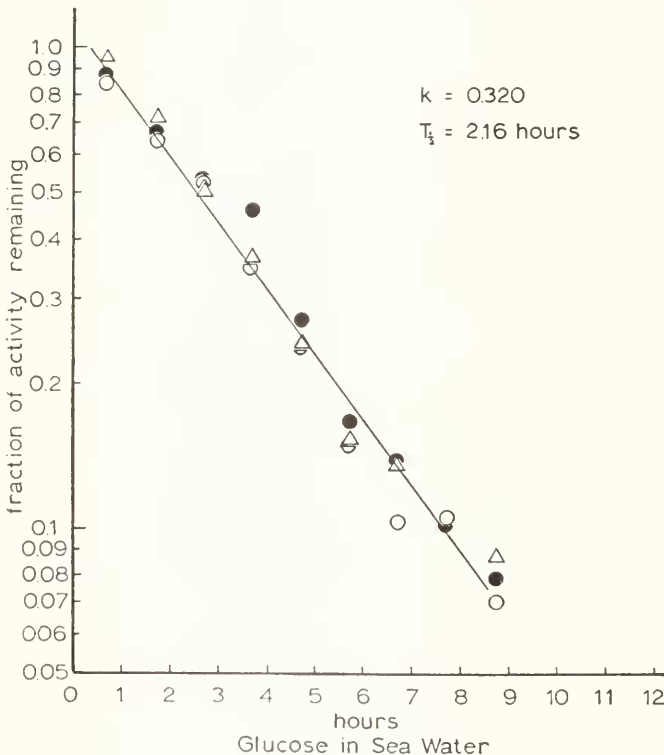


FIGURE 1. The rate of absorption of glucose-C¹⁴ from sea water by isolated digestive glands. The line represents the mean slope calculated from three experiments.

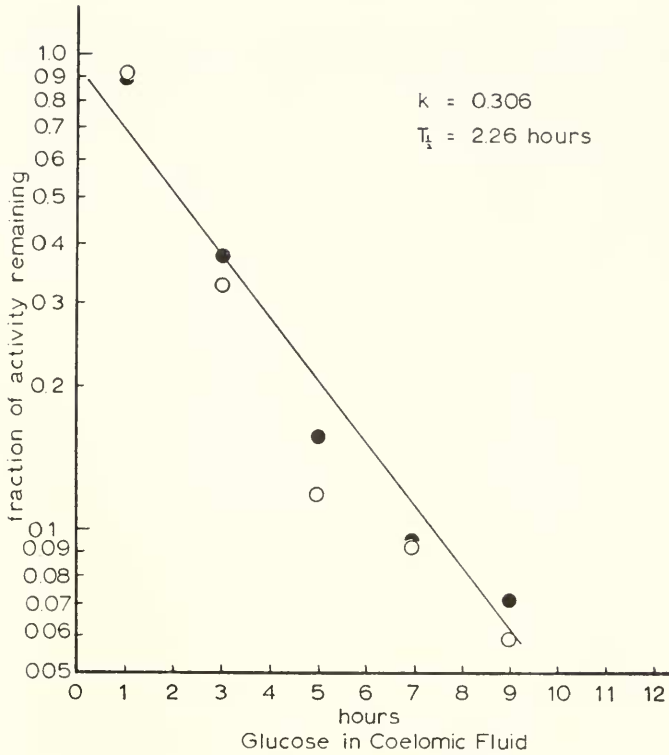


FIGURE 2. The rate of absorption of glucose-C¹⁴ from pooled, cell-free coelomic fluid by digestive glands in two experiments.

coelomic fluid (Fig. 2). In both cases the plotted data fitted a simple exponential function indicating that the rates of absorption were principally dependent on the concentrations of the tracer in the medium. The curves fitted to the data were calculated from the equation (Comar, 1955),

$$A = A_0 e^{-kt},$$

where A = the amount of activity present at time " t ";

A_0 = the amount of activity present at zero time;

k = the constant representing the fractional rate of change of " A " with time.

The time required to remove one-half the tracer, the "half value time", ($T_{1/2}$), may be calculated from the expression,

$$T_{1/2} = - \frac{2.3 \log 1/2}{k} = \frac{0.693}{k}.$$

The half value time for the absorption of glucose-C¹⁴ by digestive glands in sea water was 2.16 hours; in coelomic fluid it was 2.26 hours.

The process being observed in this and the following experiments may not be just the simple removal of a substance from a fluid medium, but rather, it is more likely the turning-over of that substance—*i.e.*, for approximately every molecule of labeled material being removed from the fluid, another unlabeled molecule moves out to take its place. Some evidence that this is the case will be found in later data. If turning-over is occurring, the “turnover time” (T_t), the time required to exchange the number of molecules equivalent to the number present in the fluid at equilibrium, would be a very useful value.

Since k represents the fractional rate of change per unit time, its reciprocal would be the turnover time:

$$T_t = \frac{1}{k}.$$

Thus, the turnover time for the digestive glands placed in sea water with glucose- C^{14} would be 3.12 hours; in the coelomic fluid, 3.27 hours. As in the animals there are ten digestive glands active in about an equivalent amount of fluid, it may be estimated that the turnover time of glucose *in vivo* would be in the order of 0.33 hour.

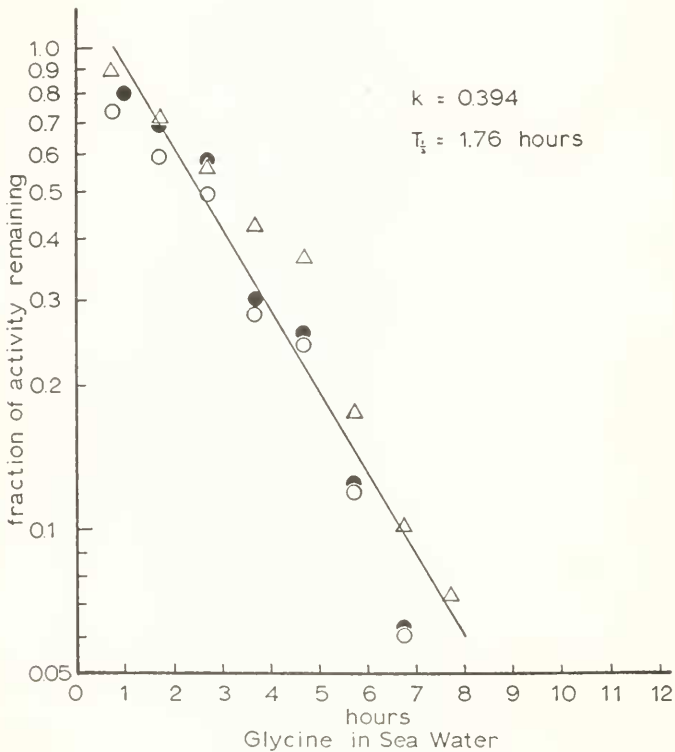


FIGURE 3. The rate of absorption of glycine- C^{14} from sea water by digestive glands in three experiments.

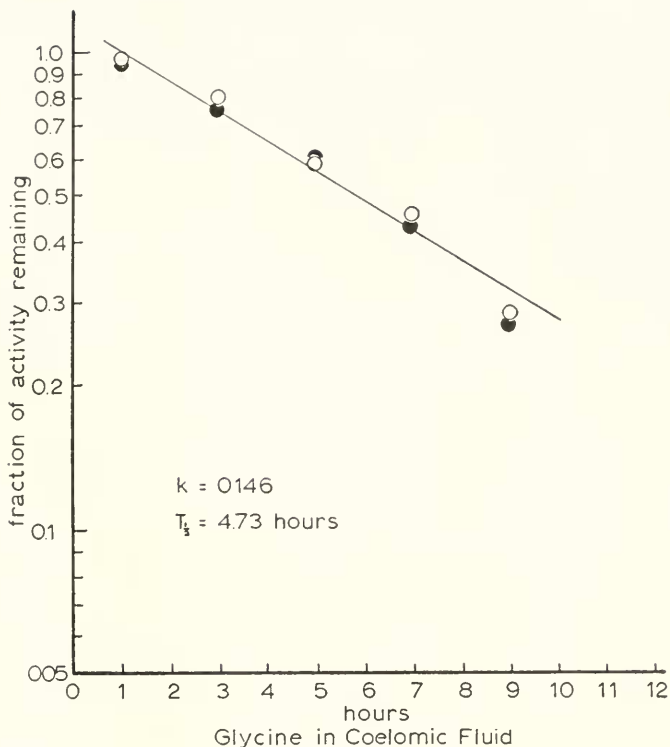


FIGURE 4. The rate of absorption of glycine- C^{14} from pooled, cell-free coelomic fluid by digestive glands in two experiments.

Absorption of glycine- C^{14}

The data obtained by incubating digestive glands with glycine- C^{14} were handled in the same manner as when glucose- C^{14} was used. With sea water as the medium, a $T_{1/2}$ of 1.76 hours was determined (Fig. 3). When the same experiment was performed in cell-free coelomic fluid, however, a much different value was obtained—a $T_{1/2}$ of 4.73 hours (Fig. 4). If the glycine that is being removed from the medium in these preparations is being replaced by unlabeled material diffusing out of the tissues, the turnover time in sea water would be 2.54 hours. In coelomic fluid it would be 6.85 hours, relating to a turnover time *in vivo* of approximately 0.69 hour.

Absorption of algal protein- C^{14} hydrolysate

Still different results were obtained when the digestive glands were incubated with algal protein- C^{14} hydrolysate (Figs. 5 and 6). There was a noticeably sharp bend at the five- or six-hour mark, correlating with the 20% of the mixture reported as "unidentified fractions" (probably less easily absorbed, higher molecular weight compounds). The slopes were calculated from the data taken before this

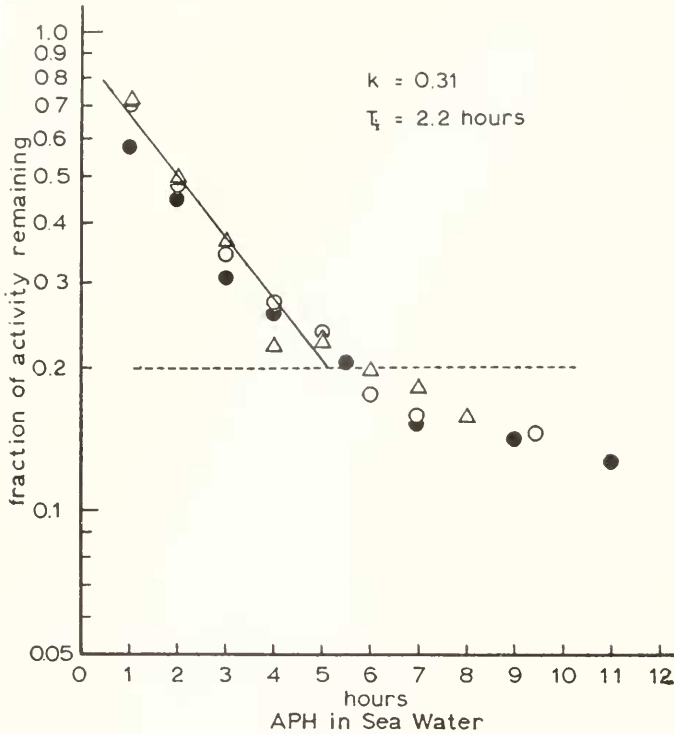


FIGURE 5. The rate of absorption of algal protein- C^{14} hydrolysate from sea water by digestive glands. The mean slope for the three experiments has been calculated only for the disappearance of the first 80% (dashed line) of the material.

point. There was little difference between the values obtained for the two media. Sea water produced a $T_{1/2}$ of 2.2 hours, while cell-free coelomic fluid gave a $T_{1/2}$ of 1.9 hours.

Again, if it is assumed that the amino acids being removed from the hydrolysate are being replaced by non-radioactive amino acids diffusing out of the digestive glands, the time required for turnover may be calculated. The turnover time in sea water turns out to be 3.22 hours. In coelomic fluid it is 2.70 hours. It is suggested, then, that an average value for the turnover of amino acids through the coelomic fluid of the starfish is approximately 0.27 hour.

Release of nitrogenous substances from digestive glands

Since the preceding experiments failed to show clearly that nutrients are given up to the coelomic fluid by the tissues, experiments were conducted to measure the release of nitrogenous materials from digestive glands placed in sea water. Figure 7 shows the appearance of total nitrogen in two such preparations. It could be expected from the experiments already presented that if nutrients were actually being given off and accumulated in the medium, they would tend to be reabsorbed at rates dependent on their concentrations. Likewise, since the digestive glands

must represent such a large reservoir of nutrients, these substances should be released at rather constant rates. These ideas may be expressed mathematically in the equation,

$$\frac{dC_t}{dt} = \lambda - kC_t$$

where λ = the rate of nutrient loss from the tissue;

k = the reabsorption rate constant;

C_t = the concentration in the medium at time "t."

The equation is separable, and by integration a working form may be obtained:

$$C_t = \frac{\lambda}{k} + \left(C_0 - \frac{\lambda}{k} \right) e^{-kt},$$

where C_0 = the initial concentration in the medium.

Using this equation, the rate of nutrient loss and the fractional rate of reabsorption have been calculated from the data, and the curves obtained by using

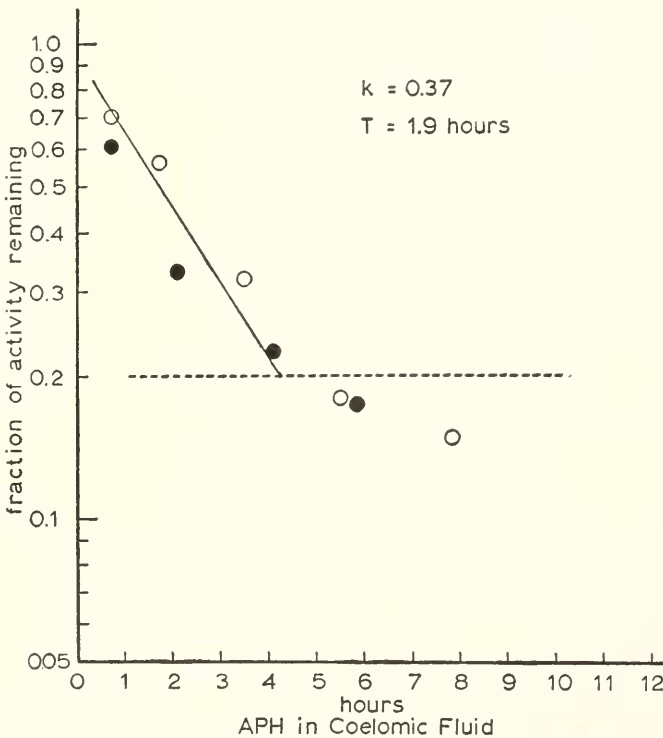


FIGURE 6. The rate of absorption of algal protein- C^{14} hydrolysate from pooled, cell-free coelomic fluid by digestive glands. The mean slope for the two experiments is from the first 80% of the activity removed.

these constants plotted in Figure 7. The concentrations expected at equilibrium would be 38.3 and 38.5 $\mu\text{g. N}$ per ml. of medium. This is within the range that has been observed to occur in the animals (Ferguson, 1964).

As some of the total nitrogen observed in the preceding experiments must represent non-nutritional free ammonia released from the metabolizing tissues,

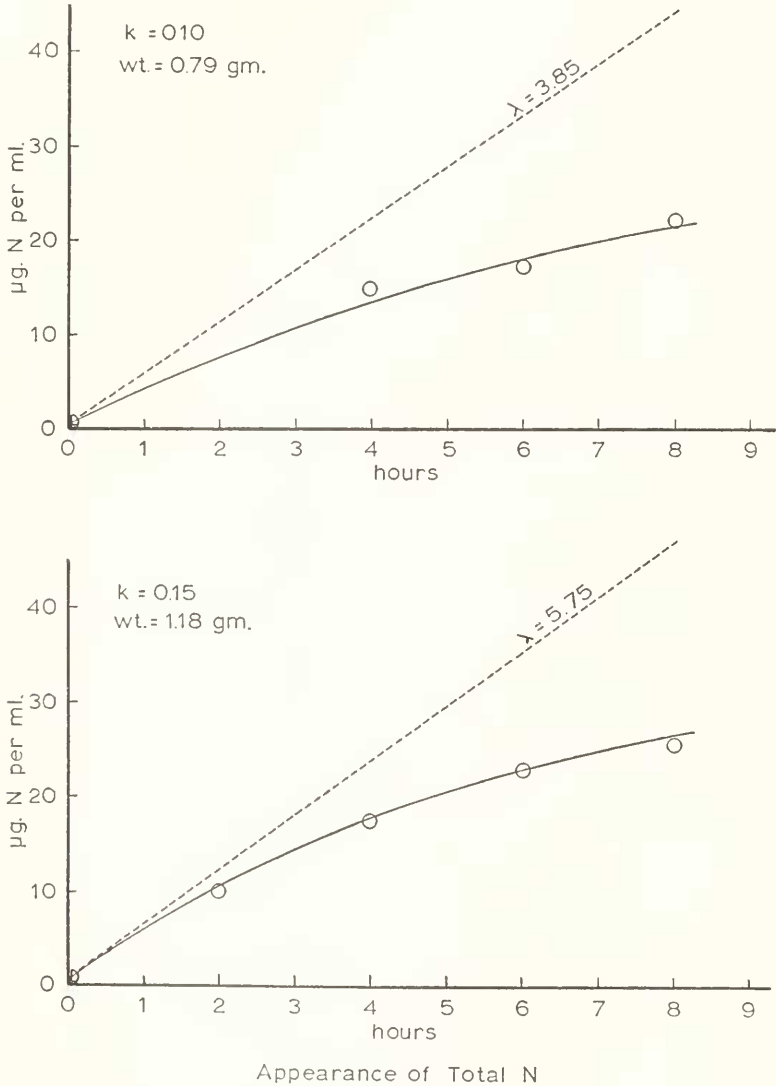
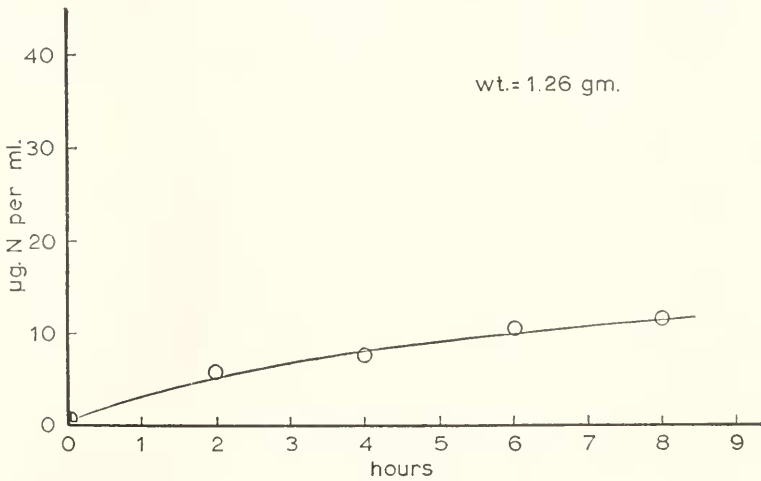
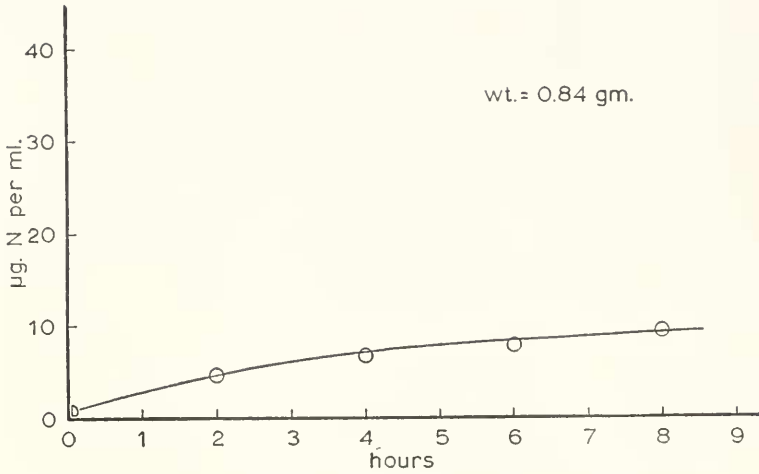


FIGURE 7. The rate of appearance in two experiments of nitrogenous substances in 10 ml. sea water containing pairs of digestive glands. The solid lines are the curves calculated to fit the data, and the dashed lines the estimated diffusion of material out of the digestive glands. The space between the solid and dashed lines represents material that has been reabsorbed by the tissues.



Appearance of Free NH_3 N

FIGURE 8. Two experiments demonstrating the rate of appearance of free ammonia in 10 ml. sea water containing pairs of digestive glands. The lines are subjective estimations of the curves.

similar experiments were performed to measure this factor. The results of two of these may be seen in Figure 8. The free ammonia values were subtracted from the comparable values of total nitrogen to give a measure of the movement of non-ammonia nitrogen into the medium. The estimates of non-ammonia nitrogen were treated in the same manner as used for the total nitrogen data. In the first case, for digestive glands weighing 0.79–0.95 gm., k was calculated as 0.25 and λ

as $3.5 \mu\text{g. N per ml. per hour}$. In the second case, for digestive glands weighing $1.18\text{--}1.23 \text{ gm.}$, k was 0.20 and λ , 3.6 . Equilibrium levels for the two cases would occur at 18 and $14 \mu\text{g. N per ml. fluid}$.

One further calculation is useful. The constant k , which represents the fraction of material removed per unit of time, can again be used to estimate the turnover time. For the non-ammonia nitrogen data, the turnover times of the two cases turn out to be 4.0 and 5.0 hours. As each of these experiments involved a pair of digestive glands, or one-fifth the total complement, the data would suggest that in the animal the time required to exchange all the nitrogenous nutrients in the coelomic fluid would be one-fifth of this, or 0.8 to 1.0 hour.

Release of nitrogenous substances from inhibited digestive glands

It has already been shown (Table IV) that the absorption of nutrients by the tissues of the starfish is probably an active process. To further confirm this, preparations containing pairs of digestive glands in sea water with $2 \times 10^{-4} M$ sodium iodoacetate were sampled and the concentrations of total nitrogen determined. The results of two such experiments are presented in Figure 9. As expected, reabsorption was inhibited and the full movement of material into the medium could be observed. The rates of release were fairly constant— $7.9 \text{ gm. N per ml. per hour}$ for the first set of glands (wt. 1.35 gm.) and 6.3 for the second

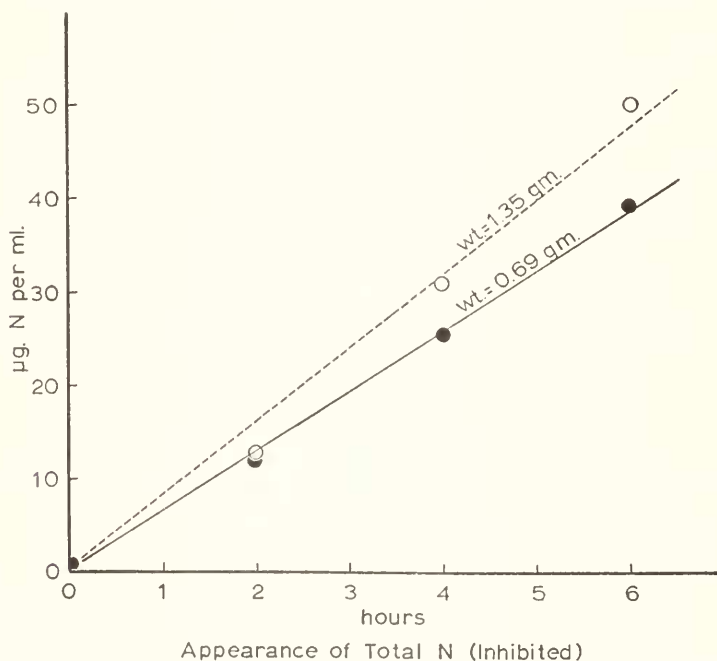


FIGURE 9. The rate of release of nitrogenous substances from pairs of digestive glands placed in 10 ml. sea water containing $2 \times 10^{-4} M$ sodium iodoacetate. The lines are the mean slopes of the two experiments.

(wt. 0.69 gm.). These values are slightly higher than those estimated from the uninhibited material, the difference possibly being due to necrosis developing in the preparations. These data, too, may be used to calculate turnover times for nitrogenous materials in the coelomic fluid of animals if the equilibrium levels (38.3 and 38.5 $\mu\text{g. N}$ per ml.) in the uninhibited total nitrogen experiments are accepted. The turnover time would be 0.97 hour in the first case and 1.22 hours in the second.

DISCUSSION

As all the organs studied demonstrated an ability to remove labeled nutrients from solutions of approximately the same concentration as found in normal coelomic fluid, one may conclude that in the living animal the different organs and tissues are continually depleting the coelomic fluid of the nutritional substance dissolved in it. This being the case, it should be expected that the concentration of nutrients measured in the body fluids of normal animals would be very low (Ferguson, 1964). The nutrients present must come from somewhere, and the only possible source is the tissues themselves. Figures 7, 8 and 9 indicate that this is the case, at least for nitrogenous materials. Nutrients are released, apparently passively, from the same tissues that are actively reabsorbing them.

Thus, these results give evidence that the organs and tissues of starfish are in a continual state of flux, with each both actively absorbing nutrients from the coelomic fluid and passively releasing them back into the same fluid. Presumably the same mechanism could serve to translocate nutrients from one region of the animal to another. It would be only necessary to have storage organs, such as the digestive glands, release material slightly more rapidly than they reabsorb it, and for the other tissues to absorb nutrients more rapidly than they release them.

There can be little doubt that these processes are of great significance in the economy of the animals, but it must be shown that they occur at rates sufficient to satisfy the metabolic needs of the starfish. The rate experiments with digestive glands are most useful in this respect. Digestive glands were chosen for this work not only because they were easy to obtain, but also because they represent the main source of nutrients appearing in the coelomic fluid. They comprise a very large proportion of the organic material in the animals, and they had previously been demonstrated to behave in a manner similar to the other tissues.

It was first noticed from these studies that nutrients were absorbed at rates dependent mainly on their concentration in the medium. A similar phenomenon was observed with injection studies by Van der Heyde (1922) (see Ferguson, 1964). It may be explained on the physical basis that as the solution becomes more dilute, fewer molecules come into contact with the tissues, and thus their extraction becomes increasingly difficult.

The turnover times calculated for these experiments were extended to give an estimate of the turnover time in the animals—20 minutes for glucose, 16 for a general mixture of amino acids, and 41 for glycine. These estimates were based on the fact that for equivalent amounts of fluid, there are ten times as many digestive glands in the animals as were used in the experiments. There is, of course, a great deal of tissue in the coelomic cavity of starfish in addition to that represented by the digestive glands. These other tissues must also be contributing to the

process, so that the actual turnover times of the different substances through the fluid would be significantly shorter than the calculated ones. But these values do have meaning in that they reflect the maximum rates at which nutrients could depart from the digestive glands to supply the needs of the other tissues.

A few simple calculations serve to show that these rates should be adequate to sustain the animals. An average starfish contains about 25 ml. of coelomic fluid with a mean amino acid concentration of probably 30 μg . of nitrogen per ml. (Ferguson, 1964). Thus 750 μg . of amino acid nitrogen could be transported from the digestive glands every 16 minutes. As there are 1440 minutes in a day, this would represent a loss of nearly 68 mg. of amino acid nitrogen in a 24-hour period. Assuming a standard nitrogen content of the amino acids of about 16%, the 68 mg. would represent almost 0.5 gm. (dry weight) of protein a day. This is an amount far greater than that which animals kept in the laboratory could be induced to ingest in several days.

A similar argument could be developed from the glucose data which would show a maximum transport capacity of about 12 mg. of glucose per day. This, also, would seem to be in excess of the quantity of this substance normally ingested.

So far, only the average rates obtained from the different classes of compounds have been discussed. It is evident from Figures 4 and 6 that the normal rates of reabsorption of similar substances need not be at all similar. Glycine- C^{14} is absorbed from the coelomic fluid much more slowly than the general group of amino acids present in the algal protein- C^{14} hydrolysate. Since the experiments demonstrate great differences in the rates of reabsorption of glycine- C^{14} from sea water, where it is the only significant amino acid, and from coelomic fluid, which contains many different amino acids, it would appear that competitive inhibition is the factor chiefly responsible, although other mechanisms cannot be ruled out. There may be in the animals certain substances, specific amino acids, sugars, *etc.*, which are turned over much more rapidly than others and which are, therefore, the most significant metabolites in the process of nutrient transport. Just what these compounds are is at present unknown, but their identification would be an important topic for future research.

Giordano, Harper and Filice (1950), using a bioassay method, observed that the coelomic fluid of the starfish, *Pisaster brevispinus*, contained only five amino acids in measurable quantities. The most important of these was glycine, making up 67.4% of the total amino nitrogen. Serine represented 5.1%, arginine 7.2%, phenylalanine 5.1%, and tryptophan 0.1%. It may be expected that the different amino acids of the starfish would diffuse into the coelomic fluid from the various body regions at rates somewhat proportional to their concentrations in the tissues. Certainly the tissues of these animals must contain more amino acids than these five. Possibly the amino acids that are actually observed in the fluid in the greatest quantity are those that are the least readily reabsorbed by the tissues. It is seen that glycine represents the most abundant amino acid in a starfish and is also found to be much more slowly absorbed *in vitro* than the algal protein- C^{14} hydrolysate amino acids. By this same hypothesis, it is predictable that serine, and to a lesser extent, arginine and phenylalanine, would show signs of being less rapidly reabsorbed by the tissues. Experiments to verify this have not yet been performed.

There is no reason why amino acids and simple sugars need be the only nutrients transported by the coelomic fluid of starfish, although these seem to be far the most important. Other compounds, proteins, polypeptides, *etc.*, are present in the fluid, albeit in fairly low concentrations. While the roles of these materials are unknown, they may be functioning in the same fashion as the amino acids, but moving at considerably slower rates.

The experiments measuring the appearance of nitrogen from isolated digestive glands seem to indicate that this is so. They show that the turnover time for all the nitrogenous substances together is several times the average for the algal protein-C¹⁴ hydrolysate amino acids. While this difference could possibly be due to experimental methods, it more likely reflects the fact that the total nitrogen turnover includes not only rapidly moving amino acids, but also more slowly absorbed, larger molecules, especially proteins. These could be materials that were simply sloughed off by the tissues and are awaiting disposal, or they could be special classes of molecules produced for very limited and specific purposes. The occurrence of this latter group of substances is partially supported by recent evidence of endocrine or neurosecretory activity in starfish (Unger, 1962).

A careful look at the absorption curves (Figs. 1 through 6) permits another interesting observation. The fractional absorption rate for many of the nutrients used did not remain exactly constant. There is a definite tendency in most of the experiments for the rate to be rather low in the first few hours, followed by varying periods, each represented by several points, of more rapid absorption. These fluctuations may be due to background variations in the counting procedure, or they may be real differences in absorption rates reflecting behavior also occurring in the animals. If the latter is the case, reabsorption of nutrients in the body would not appear to be a constant and uniformly regulated process. Concentrations of nutrients in the coelomic fluid could fluctuate widely in rather short periods of time. Such fluctuations have been observed in serial analyses of experimental animals (Ferguson, 1964).

It has been concluded that the coelomic fluid is the principal medium of nutrient transport in *Asterias*. The evidence that has led to this conclusion has been based mainly on the nature and behavior of the tissues rather than on the composition of the coelomic fluid. Thus, one might expect the same sort of nutrient movement and exchange between tissues regardless of whether they are located in the perivisceral coelom or in some other space. The fluid cavities of the water vascular system and the perihæmal system must serve similar roles and all are, no doubt, important in supplying materials to the more peripheral parts, such as the tube feet and radial nerve cords. It is highly probable that the interstitial fluids between the cells and connective tissue of the body wall and other structures serve the same purpose. There is some evidence that the connective tissue in these forms is adapted to a very unspecialized interstitial medium possibly not greatly different from the coelomic fluid (Ferguson, 1960). Further study is required to clarify the nature and function of these fluids.

SUMMARY

1. Isolated digestive glands, gonads, rectal caeca, and cardiac stomach from the starfish, *Asterias forbesi*, were shown to be able to absorb labeled amino acids and

glucose from dilute solutions in sea water and coelomic fluid. This absorption could be largely inhibited with $2 \times 10^{-4} M$ sodium iodoacetate.

2. Analyses of the rates of absorption of labeled nutrients by isolated digestive glands have led to the conclusion that these substances are very rapidly turned over through all the body fluids of the starfish. Calculations indicate that the maximum possible rate of movement of stored nutrients (amino acids) from the digestive glands to the other tissues is equivalent to nearly 0.5 gm. (dry weight) of protein per day. The maximum rate for glucose is about 12 mg. per day. These quantities probably exceed the amount of nutrients normally ingested by the animals.

3. Similar studies indicate that related compounds may differ markedly in their importance in nutrient transport. The absorption of glycine is much slower from coelomic fluid than from sea water. It is suggested that the concentrations of this amino acid build up in the body fluids because its reabsorption may be inhibited by other amino acids.

4. Studies measuring the passive diffusion of nitrogenous materials from isolated digestive glands have further verified that a rapid flux of organic nutrients occurs between the internal fluids and tissues of the starfish.

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